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(54) Title: PENCICLOVIR AND FAMCICLOVIR AND RELATED GUANINE DERIVATIVES FOR THE TREATMENT OF THE HIV-1 INFECTIONS

(57) Abstract

Use of a guanine derivative or a prodrug thereof in the treatment of viral infections.

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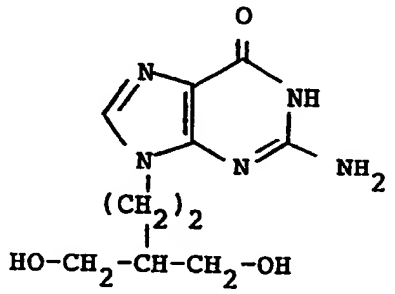
PENCICLOVIR AND FAMCICLOVIR AND RELATED GUANINE DERIVATIVES FOR THE TREATMENT OF THE HIV-1 INFECTIONS

This invention relates to a method of treatment of HIV-1 infection in humans and animals, and to the use of compounds in the preparation of a medicament for use in the treatment of such infection.

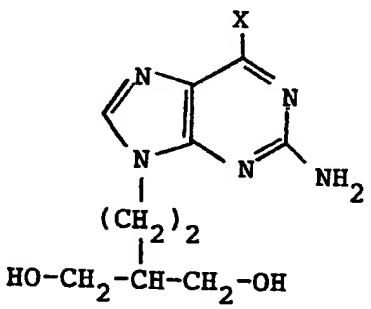
EP-A-141927 (Beecham Group p.l.c.) discloses penciclovir, the compound of formula (A):



(A)

and salts, phosphate esters and acyl derivatives thereof, as antiviral agents. The sodium salt hydrate of penciclovir is disclosed in EP-A-216459 (Beecham Group p.l.c.). Penciclovir and its antiviral activity is also disclosed in Abstract P.V11-5 p.193 of 'Abstracts of 14th Int. Congress of Microbiology', Manchester, England 7-13 September 1986 (Boyd et. al.).

Pro-drugs of the compound of formula (A) are of formula (B):



(B)

and salts and derivatives thereof as defined under formula (A); wherein X is C_{1-6} alkoxy, NH_2 or hydrogen. The compounds of formula (B) wherein X is C_{1-6} alkoxy or NH_2 are disclosed in EP-A-141927 and the compounds of formula (B) wherein X is hydrogen, disclosed in EP-A-182024 (Beecham Group p.l.c.) are preferred prodrugs. A particularly preferred example of a compound of formula (B) is that wherein X is hydrogen and wherein the two OH groups are in the form of the acetyl derivative, described in Example 2 of EP-A-182024, hereinafter referred to as famciclovir.

The compounds of formulae (A) and (B) and salts and derivatives thereof have been described as useful in the treatment of infections caused by herpesviruses, such as herpes simplex type 1, herpes simplex type 2, varicella-zoster and Epstein-Barr viruses.

It has now been discovered that these compounds have potential activity against the human immunodeficiency virus (HIV-1), in patients also infected with herpesviruses, and are therefore of potential use in the treatment of HIV infections in such patients.

This discovery is related to the ability of the triphosphate derivative of penciclovir to inhibit the RNA-directed DNA polymerase (reverse transcriptase) activity of human immunodeficiency virus type 1 (HIV-1). The reverse transcriptase of HIV-1 is a virus-encoded enzyme essential for the conversion of genomic RNA into proviral ds-DNA, and is therefore an excellent molecular target for antiviral chemotherapy.

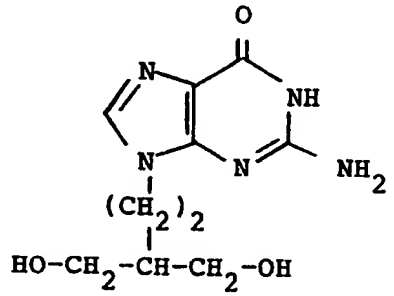
The ability of HIV to enter cells previously infected with herpesviruses is known (for example, B-lymphocytes infected with EBV[1]). The presence of both herpes and human immunodeficiency viruses in the same cell has particular consequences.

1. Penciclovir would be phosphorylated by h rpes virus-encoded thymidine kinase leading to a high level of penciclovir triphosphate[2]. The triphosphate formed is not only an inhibitor of herpes DNA polymerase, but this work indicates that it also inhibits HIV reverse transcriptase.

2. HIV replication may be enhanced by herpesvirus transactivating factors. A product of HSV, ICP-4 (infected-cell protein) can increase the initiation of HIV transcription.

3. Double infection of herpesviruses and HIV may result in phenotypic mixing and the production of 'pseudotype' HIV particles bearing herpesvirus envelope glycoproteins[3]. The packaging of HIV genomic RNA with HSV capsid proteins is also believed to occur. Either situation may lead to the infection by HIV of CD4-negative, herpesvirus-permissible cells, previously not susceptible to entry of this virus. This may also result in doubly-infected cells.

Accordingly, the present invention provides a method of treatment of HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses, which method comprises the administration to the mammal in need of such treatment, an effective amount of a compound of formula (A):



(A)

or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing.

The term 'acyl derivative' is used herein to include any derivative of the compounds of formula (A) in which one or more acyl groups are present. Such derivatives are included as pro-drugs of the compounds of formula (A) in addition to those derivatives which are per se biologically active.

Examples of pro-drugs, pharmaceutically acceptable salts and derivatives are as described in the aforementioned European Patent references, the subject matter of which are incorporated herein by reference.

A particular pro-drug of interest is 9-(4-acetoxy-3-acetoxymethylbut-1-yl)-2-aminopurine, known as famciclovir.

The compound of formula (A) may also be in one of the forms disclosed in EP-A-216459 (Beecham Group p.l.c.).

The compound of formula (A), pro-drugs, salts and derivatives may be prepared as described in the aforementioned European Patent references.

The compound, in particular, famciclovir, may be administered by the oral route to humans and may be compounded in the form of syrup, tablets or capsule. When in the form of a tablet, any pharmaceutical carrier suitable for formulating such solid compositions may be used, for example magnesium stearate, starch, lactose, glucose, rice, flour and chalk. The compound may also be in the form of an ingestible capsule, for example of gelatin, to contain the compound, or in the form of a syrup, a solution or a suspension. Suitable liquid pharmaceutical carriers include ethyl alcohol, glycerine, saline and water to which flavouring or colouring agents may be added to form syrups.

For parenteral administration, fluid unit dose forms are prepared containing the compound and a sterile vehicle. The compound depending on the vehicle and the concentration, can be either suspended or dissolved. Parenteral solutions are normally prepared by dissolving the compound in a vehicle and filter sterilising before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anaesthetic, preservatives and buffering agents are also dissolved in the vehicle. To enhance the stability, the composition can be frozen after filling into the vial and the water removed under vacuum.

Parenteral suspensions are prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the compound of the invention.

Preferred parenteral formulations include aqueous formulations using sterile water or normal saline, at a pH of around 7.4 or greater, in particular, containing penciclovir sodium salt hydrate.

As is common practice, the compositions will usually be accompanied by written or printed directions for use in the medical treatment concerned.

An amount effective to treat the virus infection depends on the nature and severity of the infection and the weight of the mammal.

A suitable dosage unit might contain from 50mg to 1g of active ingredient, for example 100 to 500mg. Such doses may be administered 1 to 4 times a day or more usually 2 or 3 times a day. The effective dose of compound will, in

general, be in the range of from 0.2 to 40mg per kilogram of body weight per day or, more usually, 10 to 20 mg/kg per day.

The present invention also provides the use of a compound of formula (A) or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing, in the preparation of a medicament for use in the treatment of HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses. Such treatment may be carried out in the manner as hereinbefore described.

The present invention further provides a pharmaceutical composition for use in the treatment of HIV-1 infections in mammals, including humans, which mammals are infected with herpesviruses, which comprises an effective amount of a compound of formula (A) or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing, and a pharmaceutically acceptable carrier. Such compositions may be prepared in the manner as hereinafter described.

The compound of formula (A) and its prodrugs show a synergistic antiviral effect in conjunction with interferons; and treatment using combination products comprising these two components for sequential or concomitant administration, by the same or different routes, are therefore within the ambit of the present invention. Such products are described in EP-A-271270 (Beecham Group p.l.c.).

It will be appreciated that the treatment of herpesvirus infected patients may include prophylaxis of herpesvirus episode attacks (suppressive treatment). In patients with

HSV infection only, suppressive treatment would probably only be given to those patients with frequent recurrences. In contrast, the aforementioned finding in relation to HIV-1, indicates the need for continuous suppressive treatment with penciclovir to all HIV-1 infected patients with herpesvirus recurrences, particularly HSV-1, HSV-2 and VZV recurrences, even though these recurrences may be infrequent.

The following biochemical data illustrate the invention.

BIOCHEMICAL DATA

Materials and Methods

Chemicals [^{3}H]dGTP (16.3 Ci/mmol) was purchased from Amersham International, Aylesbury, U.K. Template primer Poly (rC). p(dG)$_{12-18}$ (1:1, rC:dG ratio) and 2',3'-dideoxyguanosine-5-triphosphate (ddGTP) were obtained from Pharmacia Ltd., Milton Keynes, U.K. Penciclovir triphosphate (PCV-TP) was prepared in the laboratories of SmithKline Beecham Pharmaceuticals, Great Burgh, United Kingdom.

Reverse transcriptase Purified, E. coli expressed HIV-1 reverse transcriptase (RT) was supplied by the Protein Biochemistry Department of SmithKline Beecham Pharmaceuticals, Upper Merion, U.S.A. The enzyme was stored and diluted in a buffer containing 50mM Tris-HCl (pH 8.0), 10mM Hepes, 110mM NaCl, 5.7mM DTT, 0.3mM EDTA, 0.06% Triton X-100, 50% glycerol.

Assay for reverse transcriptase activity The reaction mixture for the HIV-1 RT assay contained in a volume of 120µl: 33mM Tris HCl (pH 8.0), 100mM KCl, 3.3mM $MgCl_2$, 3.3mM dithiothreitol, 0.2mM glutathione, 0.33mM EGTA (ethylene glycol-bis-(β-aminoethyl ether) N,N-tetra acetic acid), 0.033% Triton X-100, 1.02µM [^{3}H]dGTP, 0-12.39µM inhibitors ddGTP, PCV-TP, 0.3 A_{260} units/ml Poly (rC). p(dG)$_{12-18}$ and 167ng/ml RT (equivalent to 2.85nM for an equimolar mixture of p66 and p51 polypeptides). The reaction mixtures without RT were prepared in the microwells of a 96-well plate and preincubated at 37°C before the reactions were started by the addition of 20µl of the enzyme solution. The plates were then incubated for 65 minutes at 37°C. The incorporation rate was linear for the uninhibited control reaction over this time period. The reactions were terminated by the addition of 40µl of EDTA solution (0.2M,

pH7.0). The individual reaction mixtures w re transferred to a DEAE filter mat (1205-405, LKB Wallac, Finland), prew tted with 0.3M NaCl/0.03M Na citrat , using a cell harvester (1295-001, LKB Wallac). The filter mat was washed three times in the NaCl/Na citrate buffer and then once in 95% ethanol. Scintillation fluid (Beta Plate Scint, LKB Wallac) was added to the dried filter, and the reaction mixtures assayed for incorporation of radioactive dGMP in an LKB 1205 Beta Plate Liquid Scintillation Counter.

Results

The counts per minute obtained with the uninhibited control reaction mixture, and with a range of concentrations of ddGTP and PCV-TP, are shown in Tables 1 and 2 respectively. From the plots of % inhibition against concentration of inhibitor, approximate IC_{50} values were obtained as follows:-

ddGTP: 25nM
(R/S)PCV-TP: 4.3μM

Conclusion

These results indicate that the concentration of penciclovir triphosphate required to give 50% inhibition of HIV-1 reverse transcriptase is approximately 4μM. This level of PCV-TP should be obtained in the herpes-infected cell[2].

Table 1

Inhibition of HIV-1 Reverse Transcriptase by ddG-Triphosphate

Concentration of ddGTP (nM)	Incorporation of dGMP (c.p.m.)	% Inhibition
0	117892	
1	119726	0
10	89949	23.7
25	58703	50.2
50	35091	70.2
75	26132	77.8
100	22161	81.2

Table 2

Inhibition of HIV-1 Reverse Transcriptase by PCV-Triphosphate

Concentration of PCV-TP (μM)	Incorporation of dGMP (c.p.m.)	% Inhibition
0	88892	
0.124	85313	4.0
1.24	70061	21.2
3.10	51866	41.7
6.19	32921	63.0
9.29	26436	70.3
12.39	23844	73.2

References

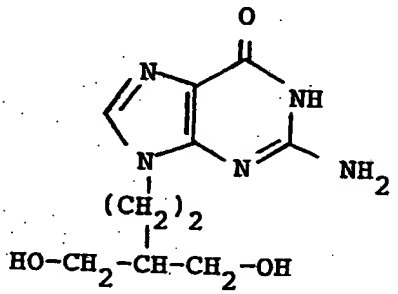
1. Complement Receptor 2 Mediates Enhancement of Human Immunodeficiency virus infection in Epstein-Barr virus-carrying B cells.
Tremblay et al., J. Exp. Med. 171, 1791 (1990).

2. Mode of action of 9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine (BRL 39123) against herpes simplex virus in MRC-5 cells.
Vere Hodge and Perkins, A.A.C., 33, 223 (1989).

3. Phenotypic mixing between human immunodeficiency virus and vesicular stomatitis virus or herpes simplex virus.
Zhu et al., J. of AIDS, 3, 215 (1990).

Claims

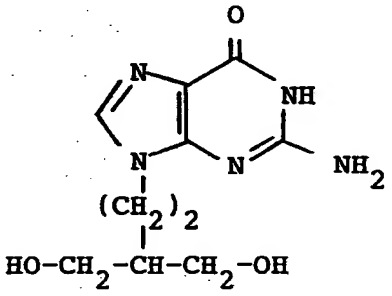
1. A method of treatment of HIV-1 inf ctions in mammals, which mammals are infected with herpesviruses, which method comprises the administration to the mammal in need of such treatment, an effective amount of a compound of formula (A):



(A)

or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing.

2. Use of a compound of formula (A):

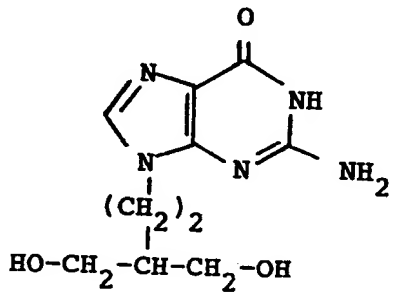


(A)

or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing; in the manufacture of a medicament for use in the

treatment of HIV-1 infections in mammals, which mammals are infected with herpesviruses.

3. A pharmaceutical composition for use in the treatment of HIV-1 infections in mammals, which mammals are infected with herpesviruses, comprising a compound of formula (A):



(A)

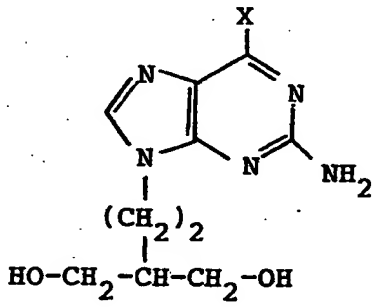
or a pro-drug, or a pharmaceutically acceptable salt, phosphate ester and/or acyl derivative of either of the foregoing; and a pharmaceutically acceptable carrier.

4. A method, use or composition according to claim 1, 2 or 3 wherein the compound is penciclovir, of formula (A).

5. A method, use or composition according to claim 1, 2 or 3 wherein the compound is the sodium salt hydrate of the compound of formula (A).

6. A method, use or composition according to claim 5 wherein the medicament is in an aqueous formulation, adapted for parenteral administration.

7. A method, use or composition according to claim 1, 2 or 3 wherein the compound is a pro-drug of the compound of formula (A), of formula (B):



(B)

or a salt or derivative thereof, as defined in respect of formula (A) in claim 1; wherein X is C_{1-6} alkoxy, NH_2 or hydrogen.

8. A method, use or composition according to claim 7 wherein the pro-drug compound of formula (B) is wherein X is hydrogen, or a derivative thereof, as defined in respect of formula (A) in claim 1.

9. A method, use or composition according to claim 8 wherein the pro-drug compound of formula (B) is famciclovir, wherein X is hydrogen and wherein the two OH groups are in the form of the acetyl derivative.

10. A method, use or composition according to claim 9 wherein the medicament is adapted for oral administration.

11. A method, use or composition according to claim 1, 2 or 3 wherein the compound administered is in a 50mg to 1g unit dose.

12. Penciclovir, or a pro-drug therefor, for use in continuous suppressive treatment of HIV patients with HSV or VZV recurr nces.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 91/01082

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/52

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	EP,A,0306844 (HOECHST AG) 15 March 1989, see the whole document	2-11
X	---	12
A	AT,B, 388500 (WELLCOME) 26 June 1989, see the abstract; claims	2-11
X	---	12
A	EP,A,0271270 (BEECHAM) 15 June 1988, see page 4, line 30 - page 8, line 33; claims	2-11
X	---	12
A	EP,A,0141927 (BEECHAM) 22 May 1985, see abstract; claims, (cited in the application)	2-11
	--- -/-	

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14-10-1991	07 NOV 1991
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

III. DOCUMENTS CONSIDERED T BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	---	12
A	Drugs Future, vol. 15, no. 4, 1990, "BRL-39123" & "BRL-42810", pages 394-395, see the whole document	2-11

X	-----	12

FURTHER INFORMATION CONTINUED FR M THE SEC ND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers * because they relate to subject matter not required to be searched by this Authority, namely:

See PCT-Rule 39.1(iv) methods for treatment of the human or animal body by surgery or therapy as well as diagnostic methods

* Claims not searched: 1
Claims searched incompletely: 4-11

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentances of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the International application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9101082

SA 49115

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 31/10/91
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0306844	15-03-89	DE-A- 3730541	06-04-89
		AU-A- 2203888	16-03-89
		JP-A- 1100123	18-04-89
AT-B- 388500	26-06-89	None	
EP-A- 0271270	15-06-88	AU-B- 606629	14-02-91
		AU-A- 8191287	02-06-88
		JP-A- 63145279	17-06-88
		US-A- 4957733	18-09-90
EP-A- 0141927	22-05-85	AU-B- 577303	22-09-88
		AU-A- 3197384	21-02-85
		JP-A- 60058982	05-04-85

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For more details about this annex : see Official Journal of the European Patent Office, No. 12/82